ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5187

On the determination of phosphates in foods

Fisheries & Oceans
LIBRARY
JAN 15 1987
BIBLIOTHÈQUE
Pêches & Océans

Y. Tonogai, and K. Tanaka

Original title: Shokuhinchuu no Rinsan'en no Teiryoo Hoo

In: J. Food Hyg. Soc. Jpn. 15(5): 331-336, 1974

Original language: Japanese

Available from:

Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1985

14 typescript pages

Secretary of State / Secrétariat d'État

90-00070/

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU BUREAU DES TRADUCTIONS

LIBRARY IDENTIFICATION – FICHE SIGNALÉTIQUE

CTFAS 5187

Translated from - Traduction de	Into - En
Japanese	English

Author - Auteur

Yasuhide TONOGAI & Kiyoko TANAKA

Title in English or French - Titre anglais ou français

On the Determination of Phosphates in Foods

Title in foreign language (Transliterate foreign characters)
Titre en langue étrangère (Transcrire en caractères romains)

Shokuhinchuu no Rinsan'en no Teiryoo Hoo.

Reference in foreign language (Name of book or publication) in full, transliterate foreign characters.
Référence en langue étrangère (Nom du livre ou publication), au complet, transcrire en caractères romains.

Shokuhin Eiseigaku Zasshi.

Reference in English or French - Référence en anglais ou français

Journal of the Food Hygiene Society of Japan.

Publisher - Editeur	DATE OF PUBLICATION / DATE DE PUBLICATION			Page Numbers in original / Numéros des pages dans l'original
Nihon Shokuhin Eisei Gakkai.	Year / Année	Volume	Issue No. / Numéro	331 - 336
Place of Publication / Lieu de publication				Number of typed pages / Nombre de pages dactylographiées
Tokyo, Japan.	1974	15	5	14

Requesting Department / Ministère-Client: DFO

Translation Bureau No. / Notre dossier nº: 2126509

Branch or Division / Direction ou Division: SJPB

Translator (Initials) / Traducteur (Initiales): KU / PS

Person requesting / Demandé par: A. T. Reid

OCT 18 1985

Your Number / Votre dossier nº:

Date of Request / Date de la demande: August 29, 1985 (received Sept. 9, 1985)

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Canada

SEC 5-111 (84-10)

Secretary of State | Secrétariat d'État

MULTILINGUAL SERVICES DIVISION – DIVISION DES SERVICES MULTILINGUES

TRANSLATION BUREAU | BUREAU DES TRADUCTIONS

Client's No.–N° du client	Department -- Ministère	Division/Branch – Division/Direction	City – Ville
	DFO	SIPB	Ottawa
Bureau No.–N° du bureau	**Language – Langue**	**Translator (Initials) – Traducteur (Initiales)**	
2126509	Japanese into English	KU / PS	OCT 18 1985

On the Determination of Phosphates in Foods

Yasuhide TONOGAI and Kiyoko TANAKA

(National Institute of Hygienic Sciences, Osaka Branch: 6, Hoenzaka-cho, Higashi-ku, Osaka)

To establish the determination methods of phosphates in foods, the following methods were examined, being followed with satisfactory results. Polyphosphates contained in foods could be separated qualitatively by thin-layer chromatography using Avicel-SF cellulose powder as adsorbent and a mixture of 0.5% pyridine—acetone solution and 20% trichloroacetic acid solution as the developing solvent. The quantity of phosphates was determined indirectly by determining the amount of molybdenum by atomic absorption spectrophotometry, after the formation of complex between phosphate and molybdate. Ion-exchange column chromatographic technique was applied for the separation of various kinds of polyphosphates contained in soft drink. These methods were applied to various kinds of foods, and from our results it was found that polyphosphates were present only in pressed ham, sausage and soft drink, and the amount of phosphates added to foods was found to be not so much.

(Received November 14, 1973)

FOREWORD

Phosphates and condensed phosphates are used in a wide variety of foods with the purpose of 'upgrading the quality', but the standard of use is not fixed except for the calcium salt.[1] Also, simple methods for the determination of phosphates in foods are not known.

It is possible that excessive intake of phosphoric acid disturbs the balance of minerals and affects the human body negatively. Therefore, we discussed the qualitative analysis of phosphates in foods by thin-layer chromatography (TLC hereafter), and the quantitative analysis by atomic absorption spectrophotometry, and checked the degree of

UNEDITED TRANSLATION
for information only
TRADUCTION NON REVISÉE
Information seulement

SEC 5-25 (Rev. 82/11)

Canada

phosphate addition to foods. Regarding soft drinks, we discussed the fractional quantitative analysis for various kinds of condensed phosphates added to them.

EXPERIMENTAL METHOD

1. Reagent and equipment

1-1. Reagent

Orthophosphate standard solution: 1.691 g of sodium phosphate ($NaH_2PO_4 \cdot 2H_2O$, special grade) was dissolved in water and filled up with water to 1000 mL (P_2O_5 concentration: 1000 ppm).

Condensed phosphate standard solution: Di- and tripolyphosphates were obtained from commercial products, and higher polyphosphates greater than tetra- were obtained from condensed phosphates prepared by heat condensation of a mixture of $NaH_2PO_4 \cdot 2H_2O$: $Na_2HPO_4 \cdot 12H_2O$ = 5 : 4 (mol ratio), which was then separated and refined by ion-exchange column chromatography[2] (Fig. 1).

Ammonia buffer solution: 0.01M ammonia water and 0.01M ammonium chloride solution were mixed and adjusted to pH 9.3.

Potassium chloride - ammonia buffer solution: Potassium chloride was dissolved into ammonia buffer solution to get various concentrations.

Molybdenum reagent: 1 g of ammonium molybdate, 5 mL of 70% perchloric acid, and 1 mL of 12M hydrochloric acid were mixed and filled up with water to 100 mL.

Reducing reagent: 1 g of stannous chloride was dissolved in 100 mL of 10% hydrochloric acid.

Cellulose powder for TLC: "Cellulose Powder MN300" made by Macherey, Nagel & Co. (MN300 hereafter), and "Avicel-SF" cellulose powder made by Asahi Kasei (Avicel hereafter) were used. 332

Anion-exchange resin: Dowex 1 x 4 (100-200 mesh), made by Dow Chemicals.

Activated charcoal: made by Merck.

All the reagents here were of special grade, and the water was distilled.

1-2. Equipment

Atomic absorption spectrophotometer: AA-1 type made by Nippon Jarrell Ash.

pH meter: Beckmann Zeromatic SS-3.

2. Qualitative analysis by TLC

Avicel SF as an adsorbent was coated in 250 μ thickness on a plate and dried at 60-80° for 30 minutes. As a developing solvent, 70 mL of 0.5% pyridine/acetone solution, 25 mL of water, and 5 g of trichloroacetic acid were mixed. Then we conducted the development, examining the extracted water from the homogenized samples. The detection was performed as follows: molybdenum reagent was sprayed against the plate, and after being heated at 80-90° for 5 minutes, reducing reagent was sprayed over it in order to get blue coloration.

3. Quantitative analysis by atomic absorption spectrophotometry

1 g of the sample was incinerated with perchloric acid and nitric acid in order to change all the various phosphoric acid compounds into orthophosphoric acid. The sample was then diluted with water so that the content of phosphoric acid (as P_2O_3) became 1-2 ppm. 5-10 mL of the sample solution was taken out into a separatory funnel and 5 mL of 5N perchloric acid solution, 2.5 mL of 5% ammonium molybdate solution, and water were added to fill it up to 25 mL. Then, 10 mL of acetic acid n-butyl was added and the solution was shaken well. By determining the amout of molybdenum in the phosphorus-molybdic acid complex, which moved into the organic layer, by

atomic absorption spectrophotometry, we obtained indirectly the amount of phosphorus in the samples. They are all shown as P_2O_5. The operating conditions for atomic absorption spectrophotometry are shown in Table 1.

4. Fractional quantitative analysis of condensed phosphates in soft drinks

After 100-200 mL of soft drinks (with carbonic acid and without fruit juice) was decolored with a small amount of activated charcoal, it was adjusted to pH 9.3 with 0.1M ammonia water. This was used as a sample solution. The sample solution was passed through Dowex 1 x 4 column (1.15 x 60 cm), which was adjusted with the ammonia buffer solution, so that various kinds of phosphoric acid ions were adsorbed. Then it was rinsed with 50 mL of ammonia buffer solution, then with 50 mL of 0.2M potassium chloride/ammonia buffer solution. The concentration of potassium chloride was then changed gradually from 0.2 to 0.35M and elution was performed at the flow rate of 40 mL/hr.[2] Mono-, di-, and tripolyphosphoric acids were eluted in this order. The remaining higher polyphosphates were eluted with 50 mL of 2M potassium chloride/ammonia buffer solution, then with 50 mL of 6N hydrochloric acid. After the 333 qualitative analysis of each eluted fraction by TLC, we grouped them into mono-, di-, tri-, and higher-than-tetra- polyphosphoric acids, and performed quantitative analysis according to the method in Section 3.

RESULTS AND DISCUSSION

1. Qualitative analysis by TLC

1-1. Discussion on the conditions of development

We discussed the combination of adsorbent and developing solvents.[3)-5)] Two kinds of adsorbent, MN 300 and Avicel, and the 5 kinds of developing solvent in Table 2, were discussed. In the case of the solvents A and

E, the separation of higher than di- and tripolyphosphoric acids was impossible. In the case of the solvent C, the separation of higher than tetra-phosphoric acids was good and the color condition at the time of development was stable in all the polyphosphates. However, it took a long time for development and the color easily faded away. These were the drawbacks. With all solvents MN 300 had a tendency to cause tailing. In the case of the combination of Avicel and the solvents B and D, and the combination of MN 300 and the solvent B, 334 the separation of higher than tetra- was relatively difficult, but the separation of up to tri- was perfect. Among them, the combination of Avicel and solvent D was most suitable from the viewpoint of the coloring degree and the development time. However, in the polyphosphoric acids higher than di-, the difference in the degree of polymerization caused different shades of blue colors at the time of development. This seemed to be due to incomplete hydrolysis of polyphosphoric acid. It therefore seemed to be inappropriate to apply densitometry on the thin layer plate for quantitative analysis.

1-2. Application to foods

In order to make a sample solution for TLC, we compared water extraction and 10% trichloroacetic acid extraction for the extraction of phosphoric acid from foods. It was found that water extraction was good enough for the extraction of phosphoric acid and that deproteinization with trichloroacetic, which entails a high risk of hydrolysis, was not necessary. Fig. 2 shows the results of TLC on several kinds of foods, using the experimental method in Section 2. In meat products such as pressed ham and sausage, and in some kinds of soft drinks, addition of polyphosphates was detected, but in most cases, spots were detected only at the position of orthophosphoric acid. Also, in the case of raw

materials for foods, spots were noted only at the position of orthophosphoric acid.

2. Quantitative analysis by atomic absorption spectrophotometry

As mentioned before, phosphates in foods are orthophosphate in most cases, and even though polyphosphates are added, they are probably reduced into lower phosphates by hydrolysis during food processing and storage. Therefore, to obtain the amount of phosphates in foods, quantitative analysis of orthophosphoric acid is good enough. The colorimetric methods in the past[6)-9)] were affected greatly 335
by coexisting substances and the analysis operation was complicated. Therefore, we discussed the indirect determination method of orthophosphoric acid by atomic absorption spectrophotometry,[10),11)] which is simpler and more accurate.

2-1. Effects of acidity at the time of extraction of phosphomolybdic acid complex

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

Effects of concentration of perchloric acid at the time of the extraction of phosphomolybdic acid complex were discussed. The results are shown in Fig. 3. The extraction rate became almost constant in the range of 0.25-1.75 N. Therefore, slightly different concentration of perchloric acid in each sample at the time of incineration does not affect the extraction rate. The concentration of perchloric acid at the time of extraction was consequently set at 1 N. The fluctuation coefficient of the extraction rate was 1.3%. Also, arsenic principal metal which is considered to hinder the determination of phosphorus by forming heteropolyphosphates with molybdenum, like phosphorus, is determined by the same extraction method as in phosphoric acid. As shown in Fig. 3, the extraction rate of arsenic is low, and

the coexistence of about 50 times more arsenic hardly affects the determination of phosphoric acid.

2-2. Calibration curve and recovery of added phosphates.

Fig. 4 shows the calibration curve of the standard succession of phosphates in the range of 0-1 ppm, determined by atomic absorption spectrophotometry described in Section 3. It shows a good straight line up to 1 ppm as P_2O_5. We also discussed the recovery rate of 1-5 mg of phosphoric acid (P_2O_5) added to each 1 g of various foods. Table 3 shows that the recovery rates are mostly quantitative in all cases, and the effects of various coexisting substances in foods were negligible.

2-3. Content of phosphates in foods.

Table 4 shows the content of phosphates in typical foods determined by the experimental method in Section 3. However, these samples were used without drying. Water content, therefore, has to be considered in the determined values. In order to know the amount of phosphates added to foods, we determined the amount of phosphoric acid naturally contained in raw food materials. The results are shown in Table 5. The amount was as high as in foods shown in Table 4. These results show that the quantity of phosphates added to foods is not so much as to be harmful to human bodies at present. However, it is difficult to subtract the average value of natural phosphates from the added amount because the latter is small. Therefore, in order to know the added amount, it is necessary to use the natural phosphate content in each raw material before phosphate is added as blank values. However, all the phosphates in soft drinks which do not contain natural fruit juice are considered to be added artificially. We therefore discussed the fractional determination method for them.

3. Discussion on the fractional quantitative analysis of condensed phosphates in soft drinks.

3-1. Elimination of coexisting substances.

Additives in soft drinks without natural fruit juice are sugar, acids, flavour, and colors. Most of them are hardly adsorbed in resin. They can therefore be eliminated easily while passing through the column. However, colors were strongly adsorbed and caused the elution rate of phosphoric acid from resin to be lowered to 70%. Therefore, the samples with colors were decolored first with a small amount of activated charcoal. The loss of phosphoric acid by the treatment was negligible.

3-2. Quantitative analysis of polyphosphates separated by ion-exchange resin column.

336

The samples were adjusted to pH 9.3 and passed through a column which was filled with resin adjusted pH 9.3 beforehand. All the phosphates in the samples were adsorbed completely and a good recovery rate of 95-103% was obtained. The pattern of separation and elution was relatively faster than in the standard solution of polyphosphates in Fig. 1, but there was not much difference. Then, in order to check the hydrolysis of polyphosphates during development in column chromatography, we performed the ion-exchange chromatography, using the experimental method described in Section 4, with standard solutions of mono-, di-, and triphosphoric acids. The results are shown in Table 6. It was found that 1.5% of di-, and 7% of tri- were hydrolyzed during passage through the column. Therefore, the values of each phosphate determined in Section 3-3 are slightly affected by hydrolysis (Table 7).

3-3. Application.

Table 7 shows the results of fractional quantitative analysis of

several kinds of commercial soft drinks sold on the market, using the experimental method of Section 4. Only orthophosphoric acid was detected in cokes, and only a small amount of orthophosphoric acid was detected in orange juice. Polyphosphoric acids were found only in grape juice. It seemed that the addition of polyphosphoric acids has some effect in giving a flavour of grapes.

SUMMARY

1) The results of the qualitative analysis of phosphates in foods by TLC show that polyphosphates were added to meat products and some kinds of soft drinks, but that only orthophosphoric acid was added to most of the foods.

2) Quantitative analysis by atomic absorption spectrophotometry, which is simple and accurate, was discussed and used to determine the content of phosphoric acid in foods and in raw food materials. The results show that not much difference in content existed between them.

3) Fractional quantitative analysis of polyphosphates added to soft drinks, using ion-exchange column chromatography, provided good results.

Before finishing this article, we would like to extend our appreciation to Mr. M. Keida, Head of the Food Dept., and Mr. Y. Nomura, Former Head of the Food Dept., Osaka Branch, National Institute of Hygienic Sciences, who gave us advice. Also, we have to mention here that this study is a part of the special study for 1972 by the National Institute of Hygienic Sciences (Safety studies on various substances in foods).

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement

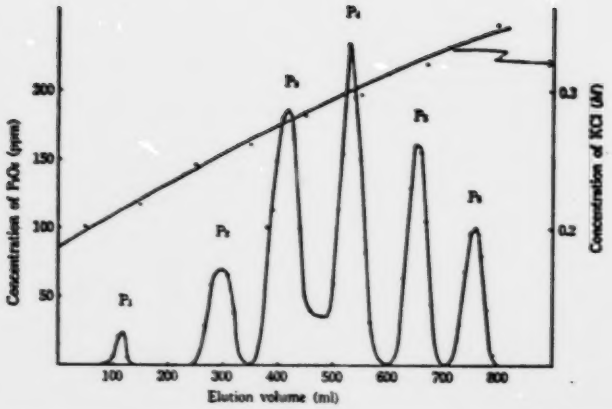

Fig. 1. Elution curve of polyphosphates by ion-exchange chromatography: Resin, Dowex 1 x 4 (100~200 mesh), Cl^- form; eluent, KCl solution, pH 9.3; flow rate, 40 ml/hr; sample, a mixture of polyphosphates prepared by heat condensation of monobasic sodium phosphate and 12 hydrate of dibasic sodium phosphate.

Table 1. Operating Conditions for the Determination of Phosphate by Atomic Absorption Spectrophotometry

Light source	Mo hollow cathode lamp
Wavelength	313.4 nm
Lamp current	20 mA
Burner type	Slot burner
Flow rate	N_2O: 6 L/min
	C_2H_2: 4 L/min

Table 2. Solvent System for Thin-Layer Chromatography

Solvent		Solvent system				
		A	B	C	D	E
Methanol	(ml)	60	75			180
Dioxane	(ml)	30		70		60
Isopropanol	(ml)					35
Isopropanol soln.[a]	(ml)	30	20			
Acetic acid (100%)	(ml)					4
Acetic acid soln. (20%)	(ml)	8	6			
Trichloroacetic acid (TCA)	(g)				5	
TCA soln. I[b]	(ml)	40	25			
TCA soln. II[c]	(ml)			30		
TCA soln. III[d]	(ml)					32
Pyridine—acetone soln.[e]	(ml)				70	
Water	(ml)				25	21

[a] 700 ml of isopropanol plus 100 ml of water
[b] 125 g of TCA plus 32 ml of 28% ammonia water, filled up with water to 1 L
[c] 160 g of TCA plus 8 ml of 28% ammonia water, filled up with water to 1 L
[d] 375 g of TCA plus 96 ml of 28% ammonia water, filled up with water to 1 L
[e] 199 ml of acetone plus 1 ml of pyridine

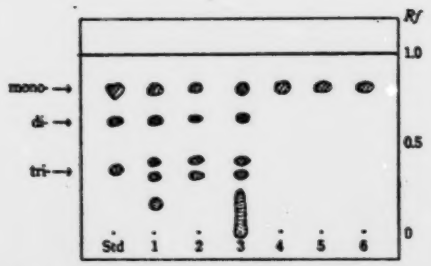

Fig. 2. Thin-layer chromatogram of various phosphates in foods: Adsorbent, Avicel-SF (cellulose powder); developing solvent, a mixture of 70 ml of 0.5% pyridine/acetone and 25 ml of 20% trichloroacetic acid; Std, standard; 1, pressed ham; 2, sausage; 3, soft drink; 4, cheese; 5, "Kamaboko" (fish-paste product); 6, noodle.

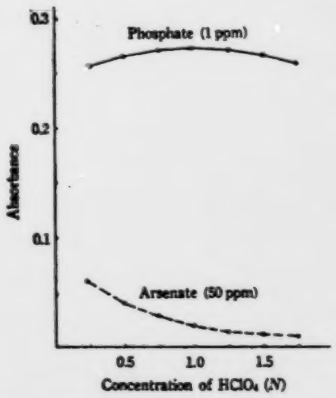

Fig. 3. Effect of perchloric acid on the extraction of phosphate and arsenate

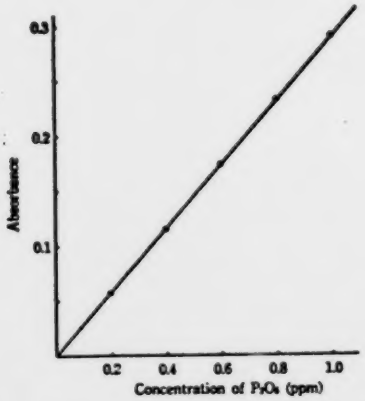

Fig. 4. Calibration curve of phosphate by atomic absorption spectrophotometry

Table 3. Recovery of Phosphate Added to Various Foods

Food (1 g)	Content (mg)	Recovery[a]		
		Added[b] (mg)	Recovered (mg)	%
Margarine	0.21	1.00	0.98	98.0
Noodle	0.90	1.00	1.00	100.0
"Kamaboko"	2.72	1.00	1.05	105.0
Bean paste	4.40	5.00	5.04	100.7
Pressed ham	4.96	5.00	4.62	92.4
Cheese	23.2	5.00	4.80	96.0

[a] Mean value of ten trials
[b] Sodium monophosphate solution was used

Table 4. Distribution of Phosphates in Processed Foods

Food	Content[a] (mg/g)	Food	Content[a] (mg/g)
Soft drink	0.40	Soy sauce	3.52
Ketchup	0.94	Pressed ham	4.65
"Chikuwa"[b]	1.10	Cracker	4.80
Bean curd	1.60	Sausage	5.50
Mayonnaise	2.40	Frozen bean curd	18.50
Bread	2.86	Parmesan cheese	23.20

[a] Mean value of three trials
[b] A kind of fish-paste product

Table 5. Distribution of Phosphates in Natural Foods

Food	Content[a] (mg/g)	Food	Content[a] (mg/g)
Wheat flour	1.68	Beef	4.10
Polished rice	2.48	Pork	4.30
Unpolished rice	6.50	Fish meat	12.70
"Azuki" bean	9.50	Egg yolk	13.30
Soybean	11.90	Sesame seed	13.60

a) Mean value of three trials

Table 6. Recovery of Polyphosphates by Ion-exchange Chromatography

Phosphates	Added (mg)	Eluted (mg)		
		Mono-	Di-	Tri-
Monophosphate	33.6	33.5 (99.6)*	—	—
Diphosphate	31.5	0.5 (1.5)*	31.8 (101)*	—
Triphosphate	32.1	1.5 (4.7)*	0.8 (2.3)*	30.0 (93.0)*

* Percentage of recovery

Table 7. Determination of Polyphosphates in Soft Drinks

Sample	Content of total phosphate (ppm)	Composition of phosphates[a] (%)			
		Mono-	Di-	Tri-	Poly-[b]
A	380	100	—	—	—
B	390	100	—	—	—
C	430	100	—	—	—
D	320	4.6	8.2	4.1	83.1
E	160	33.6	29.4	30.6	6.2

a) Mean value of three trials
b) Total of polyphosphates containing more than three phosphorus atoms

References

1) Editorial Committee for Comments on the Regulations on Food Additives, ed. : Dai 2-han Shokuhin Tenkabutsu Kouteisho Chuukai(Comments on the Regulations on Food Additives, Second Edition), p. 959 (1968) Kanahara Shuppan.

2) Ohashi, S., Tsuji, N., Ueno, Y., Takeshita, M., Muto, M.: J. Chromatog., 50, 349 (1970).

3) Rössel, T.: Z. Anal. Chem., 197, 333 (1963).

4) Wyler, O. D.: Fleischwirtschaft, 52, 175 (1972).

5) Neraal, R., Hämm, R.: *ibid.*, 52, 1171 (1972).

6) Ishii, D., Takeuchi, T.: Bunseki Kagaku (Japan Analyst), 10, 1391 (1961).

7) Maekawa, S., Kato, K.: ibid., 17, 597 (1968).

8) Boltz, D. F. ed.: "Colorimetric Determination of Nonmetals", Interscience Publishers, (1958) Chap. II.

9) Ohashi, S., ed.: Muki Kagaku Zensho IV-6 Rin (Complete Book on Inorganic Chemistry IV-6, Phosphorus), p. 32 (1965) Maruzen.

10) Ishibashi, M., Tabuse, M.: Bunseki Kagaku (Japan Analyst), 8, 588 (1959).

11) Kumamaru, T., Otani, T., Yamamoto, Y.: Bull. Chem. Soc. Japan, 40, 429 (1967).